Enzo

MTB Microplate Hybridization Assay

Cat. No. 46340

•For Research Use Only

INTENDED USE

The ENZO MTB Microplate Hybridization Assay Kit provides materials for the detection of *Mycobacterium tuberculosis* complex (MTB) nucleic acid in a microplate assay using a biotin-based detection system. Organisms of the MTB complex, which consists of *M. tuberculosis*, the most clinically significant mycobacterial pathogen, *M. bovis, M. bovis BCG, M. africanum* and *M. microtii,* are responsible for significant morbidity and mortality in humans. The assay yields negative results for 25 other mycobacterial species, including *M. avium, M. intracellulare, M. kansasii, M. chelonae* and 63 other bacterial species, including a variety of respiratory pathogens.

SUMMARY, EXPLANATION AND PRINCIPLE

The ENZO MTB Microplate Hybridization Assay is a non-radioactive, colorimetric hybridization procedure performed in microwell format. MTB DNA can be assayed indirectly in procedures employing target amplification, or culture, or it can be assayed directly if there is sufficient target DNA present. The detection procedure has been developed for use with a streptavidin-horseradish peroxidase complex to visualize the presence of biotin-labeled probes.

The ENZO non-radioactive procedure involves pretreatment of the sample to denature the DNA, followed by hybridization of the DNA to a well-bound capture probe. The captured MTB DNA is then hybridized to a signal probe. The resulting hybrid is reacted with a biotin-labeled oligomer, and the biotin is detected using a streptavidin-horseradish peroxidase detection system. A positive reaction is indicated by the appearance of bright yellow color which can be measured by a microplate reader. Using this format, 10^7 to 10^8 copies of target sequences can be detected.

STORAGE

Store the kit at 2° - 8°C. DO NOT FREEZE. When used and stored as directed, the kit is stable until the expiration date indicated on the box.

U.S. Patent Nos. 4,711,955, 4,994,373, and 5,328,824, EP 0 063 879 B1, EP 0 117 440 B1 and Patents Pending

REAGENTS AND MATERIALS PROVIDED

The ENZO MTB Microplate Hybridization Assay Kit provides reagents for testing 96 samples in a microwell strip format.

Vial 1 **Denaturation Reagent, 3 ml**
Dilute alkaline solution containing indicator

Vial 2 **Hybridization Buffer, 10 ml**
Buffered sodium chloride/EDTA containing formamide and hybridization enhancers

Vial 3 **MTB Signal Probe, 6 ml**
Modified MTB-specific DNA probe in buffered sodium chloride/EDTA containing formamide, hybridization enhancers and indicator

Vial 4 **Linker, 6 ml**
Modified poly-dA in buffered sodium chloride/sodium citrate containing detergent

Vial 5 **20X Rinse Buffer, 25 ml**
Buffered sodium chloride/sodium citrate containing detergent

Vial 6a **10X Detection Reagent, 1.0 ml**
Streptavidin-horseradish peroxidase complex in buffered sodium chloride, stabilizer and detergent

Vial 6b **Detection Buffer, 10 ml**
Buffered sodium chloride/EDTA containing stabilizer and detergent

Vial 7a **Chromogen Reagent, 1.5 ml**
5 mg/ml tetramethylbenzidine (TMB) in solvent

Vial 7b **Reaction Buffer/Substrate Reagent, 15 ml**
Dilute hydrogen peroxide in citrate phosphate buffer

Vial 8 **Stop Solution, 12 ml**
Dilute acid solution

Vial 9 **MTB Positive Control, 100 µl**
DNA carrying MTB complex sequences

Precoated Microwells, 6 microwell strips (2 x 8) in a strip holder, 96 wells
Microwells coated with MTB-specific capture probe

Plate Sealer, 1

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate shaker
- Precision pipets capable of delivering volumes of 5 µl to 1 ml
- Polypropylene microtubes
- Microplate reader (*optional*)
- Sterile distilled water
- Tris-EDTA buffer (10mM Tris-HCl, 1mM EDTA), pH 7.5, for use when diluting samples prior to assay

WARNINGS

- **For RESEARCH use only! Not to be used for *in vitro* diagnostic purposes.**
- Read all instructions prior to performing this assay.
- Wear disposable gloves while handling kit reagents and specimens. Wash hands thoroughly after handling.
- Do not smoke, eat, drink or apply cosmetics in areas in which specimens or kit reagents are handled.
- Do not pipet by mouth.
- Use a separate disposable pipet or pipet tip for each transfer of sample to avoid cross-contamination.
- Ensure that all test samples and controls are subjected to the same processing and incubation times. Once the assay has been started, unless otherwise indicated, all subsequent steps should be completed without interruption and within the time limits recommended by the procedure.
- **Chemical Hazards.** The following reagents should be handled with care as detailed below.

  Denaturation Reagent (Vial 1) contains sodium hydroxide which is poisonous and can cause severe burns. Do not ingest or breathe vapor and avoid contact with skin, eyes or clothing. Wash after handling.

  Hybridization Buffer (Vial 2) and MTB Signal Probe (Vial 3) contain formamide which is a teratogen and an irritant. Skin contact should be avoided. Specifically, pregnant workers should avoid any exposure.If skin contact is made, wash thoroughly with soap and water.

  Chromogen Reagent (Vial 7a) contains dimethylformamide (DMF). Use glass and/or polypropylene pipets and containers when diluting. DMF can cause skin irritation. If skin contact is made, wash thoroughly with soap and water.

  Stop Solution (Vial 8) contains dilute sulfuric acid which is poisonous and can cause severe burns. Do not ingest or breathe vapor and avoid contact with skin, eyes or clothing. Wash after handling.

ASSAY CONSIDERATIONS

- The ENZO MTB Microplate Hybridization Assay Kit contains sufficient reagents and materials to analyze 96 samples, including positive and negative controls.
- If the test is to be performed on diluted material, Tris-EDTA buffer (10mM Tris-HCl, 1mM EDTA), pH 7.5, should be used as the diluent.
- Each time an assay is run, include appropriate positive and negative controls in parallel with the samples to be analyzed.

PREPARATION OF REAGENTS

1X Rinse Buffer: Dilute the 20X Rinse Buffer (Vial 5) 1:20 in sterile distilled water. Once diluted, the buffer must be kept at 2° - 8°C when not in use and must be used within one week of preparation.

1X Detection Reagent: Dilute the 10X Detection Reagent (Vial 6a) 1:10 in Detection Buffer (Vial 6b). Gently mix. Use within two hours.

Chromogen/Substrate Mixture: Using glass or polypropylene pipets and mixing container, prepare Chromogen/Substrate Mixture by adding 100 µl of Chromogen Reagent (Vial 7a) per 1 ml of Reaction Buffer/Substrate Reagent (Vial 7b). Mix well and keep in the dark. This solution must be prepared fresh for each run.

SAMPLE PREPARATION

NOTE: Warm all reagents and test components to room temperature prior to beginning the assay.

STEP 1: Pipet 30 µl of **Denaturation Reagent** (Vial 1) into each of a sufficient number of polypropylene microtubes to accommodate the number of samples and controls to be assayed.

STEP 2: To the tubes prepared in step 1, add 10 µl of each sample to be tested, including a **Positive Control** (Vial 9) and a negative control (TE buffer).

STEP 3: Incubate the tubes (samples and controls) at room temperature for 15 minutes to denature the target nucleic acid sequences.

HYBRIDIZATION/DETECTION PROCEDURE

NOTE: a. All steps are performed at room temperature. Room temperature for the purposes of this assay is defined as 23-27°C. The assay may be performed at fixed temperatures within this range. As in any temperature-dependent reaction, the quantitative values obtained will depend on the temperature at which the reaction is performed.

b. Do not allow the wells to dry out between steps.

c. Secure strips with strip retainer or adhesive tape.

STEP 4: Rinse each microwell 5 times with **1X Rinse Buffer** (diluted from 20X solution, see **Preparation of Reagents**) using 200 µl each rinse. Flick the contents of the microwells into a suitable liquid waste container and blot off the residual liquid on an absorbent surface, *e.g.*, stacked paper towels, after each wash.

STEP 5: Add 80 µl of **Hybridization Buffer** (Vial 2) to each well. Then, add 20 µl **denatured samples** to the appropriate wells.

STEP 6: After adding all samples to the microwells, seal the plate/strip(s) and incubate with shaking for 120 minutes to allow hybridization of target DNA to the well-bound capture probe. The samples will turn from blue to yellow.

STEP 7: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Then, add 50 µl of **Signal Probe** (Vial 3) to each well and incubate with shaking for 15 minutes.

STEP 8: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Rinse each microwell 5 times with 200 µl of **1X Rinse Buffer**, flicking the liquid and blotting in between each wash. Then, add 50 µl of **Linker** (Vial 4) to each well and incubate with shaking for 10 minutes.

STEP 9: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Then, add 50 µl of **1X Detection Reagent** (diluted from 10X solution, see **Preparation of Reagents**) to each well and incubate with shaking for 15-20 minutes.

STEP 10: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Rinse each microwell 5 times with 200 µl of **1X Rinse Buffer**, flicking the liquid and blotting in between each wash. Then, add 100 µl of **Chromogen/Substrate Mixture** (prepared from Vials 7a and 7b, see **Preparation of Reagents**) to each well and incubate in the dark for 15 minutes. Positive samples will turn blue.

STEP 11: Stop the color reaction by adding 100 µl of **Stop Solution** (Vial 8) to each well. Positive samples will turn from blue to yellow.

INTERPRETATION OF RESULTS

- A positive result appears as a blue color which develops after addition of the Chromogen/Substrate Mixture. The blue color changes to yellow upon addition of the Stop Solution.
- Results may be quantified by reading OD at 450 nm using a microplate reader. The positive control should give an OD reading of at least 0.5 when the assay is performed at 23-24°C. When the assay is performed at higher temperatures the positive control will give a higher OD reading.

For Technical Assistance call ENZO:
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